RESEARCH PAPER

Design, Development and Optimization and In Vitro Evaluation of Fisetin Loaded Phytosomes by Using Central Composite Design

Mounika Yadav.A[1], Siva Sankar Reddy. L[2], Dastagiri Reddy .Y[3*]

[1]Department of Industrial Pharmacy, Santhiram College of Pharmacy, Nandyal, Andhra Pradesh, India
[2]Department of Pharmaceutical Analysis, Santhiram College of Pharmacy, Nandyal, Andhra Pradesh, India
[3*]Department of Pharmaceutics, Santhiram College of Pharmacy, Nandyal, Andhra Pradesh, India. Address for correspondence: Dr. Y. Dastagiri Reddy, Professor, Department of Pharmaceutics.
Email: dastu1984@gmail.com. Cell No. 9703980570

ABSTRACT

Purpose: This study aimed to develop and optimize fisetin-loaded phytosomes to improve solubility and bioavailability using phosphatidylcholine as lipid and Kolliphor P188 as stabilizer through Response Surface Methodology with Central Composite Design.

Method: Phytosomes were prepared by the thin film hydration method. Independent variables included fisetin (X1), phosphatidylcholine (X2), and Kolliphor P188 (X3), while responses were entrapment efficiency (Y1), vesicle size (Y2), and drug release (Y3). Drug–excipient compatibility was evaluated using FTIR. Prepared formulations were characterized for entrapment efficiency, vesicle size, zeta potential, in vitro release, and morphology by TEM.

Results: FTIR confirmed absence of interaction between components. Formulation variables significantly influenced all responses. A formulation containing 572 mg fisetin, 1536 mg phosphatidylcholine, and 100 mg Kolliphor P188 showed enhanced release. The optimized batch (429 mg fisetin, 1152 mg phosphatidylcholine, 125 mg Kolliphor P188) exhibited entrapment efficiency of 92.4%, vesicle size of 162.4 nm, and drug release of 91.36% within 12 hours. Release kinetics followed zero-order model with non-Fickian diffusion mechanism.

Conclusion: Optimized phytosomes demonstrated high entrapment, nanoscale size, and sustained release, indicating improved delivery of fisetin. The study confirms that statistical optimization using CCD effectively enhances formulation performance.

Key words: Fisetin, Phytosomes, Central composite design, Transmission Electron Microscopy, Phosphotidyl Choline, Kolliphor P188

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Introduction

The therapeutic potential of plant-derived flavonoids has attracted significant attention in recent years due to their diverse pharmacological activities, including antioxidant, anti-inflammatory, and anticancer properties. Among these, fisetin (3,3′,4′,7-tetrahydroxyflavone) is a naturally occurring flavonol abundantly present in fruits and vegetables such as strawberries, apples, and onions. It exhibits promising biological activities including neuroprotective, anticancer, and anti-aging effects. However, the clinical applicability of fisetin is severely limited by its poor aqueous solubility, low bioavailability, and rapid metabolism, which restrict its therapeutic efficacy[1,2]

To overcome such limitations, lipid-based nanocarriers have been widely explored. Among them, phytosomes—a novel drug delivery system—have demonstrated remarkable potential in improving the bioavailability of phytoconstituents. Phytosomes are molecular complexes formed between a phytoconstituent and phospholipids such as phosphatidylcholine (PC), resulting in enhanced membrane permeability, improved solubility, and better pharmacokinetic profiles[3,4]. The amphiphilic nature of phosphatidylcholine facilitates the formation of stable vesicular systems that mimic biological membranes, thereby enhancing drug absorption and cellular uptake.

In addition to phospholipids, the incorporation of suitable stabilizers plays a crucial role in improving the stability and performance of phytosomal formulations. **Kolliphor P188 (Poloxamer 188)** is a non-ionic surfactant widely used as a stabilizer due to its ability to reduce aggregation, enhance dispersion stability, and improve drug release characteristics. Its inclusion in phytosomal systems

contributes to reduced vesicle size and enhanced entrapment efficiency[5].

The preparation method significantly influences the physicochemical characteristics of phytosomes. Among various techniques, the **thin film hydration method** is one of the most commonly employed approaches due to its simplicity, reproducibility, and ability to produce uniform vesicles. In this method, the drug and phospholipid are dissolved in an organic solvent, followed by solvent evaporation to form a thin film, which is subsequently hydrated to yield vesicular phytosomes [4]

Optimization of formulation variables is essential to achieve desirable characteristics such as high entrapment efficiency, optimal vesicle size, and controlled drug release. Traditional optimization methods are often time-consuming and fail to capture interaction effects between variables. Therefore, Response Surface Methodology (RSM), particularly Central Composite Design (CCD), has been widely adopted as a statistical and mathematical tool for formulation optimization. CCD enables systematic evaluation of multiple variables and their interactions with a reduced number of experimental runs, providing predictive models for optimization[3,4].

In the present study, fisetin-loaded phytosomes are developed using phosphatidylcholine as the lipid component and Kolliphor P188 as a stabilizer, employing the thin film hydration technique. The formulation is optimized using Central Composite Design under Response Surface Methodology, where independent variables include the concentration of fisetin (X1), phosphatidylcholine (X2), and Kolliphor P188 (X3). The effects of these variables are evaluated on critical response parameters, namely entrapment efficiency (Y1), vesicle size (Y2), and in vitro drug release (Y3). This systematic approach aims to develop an optimized phytosomal formulation with enhanced physicochemical properties and improved therapeutic performance.[5]

MATERIALS AND METHODS

Fisetin was purchased from Hebei Feilaimi Technology, China. Phosphatidylcholine was procured from Yarrow chem Products Mumbai, India, Kolliphor P188 was obtained as gift sample from BASF Mumbai, India. Solvents like Methanol (HPLC grade) and Water for HPLC glycol bought from Merck chemicals, Mumbai.

Preparation of Phytosomes by Thin film Hydration Technique

Weigh 286 mg of Fisetin and dissolve in 25ml of ethanol. Dissolve 100mg of surfactant P188 in ethanol. Weigh 768mg of PC and dissolve in 30 ml of chloroform. Add surfactant solution into Fisetin solution completely until it becomes homogenous mixture. Now take round bottom flask add both the solutions i,e Fisetin & PC.Continue the process until a thin film layer is formed by maintaining the temperature 55°C at 30 rpm. Now collect the product and observe under the inverted microscope.[6]

Table:1. Formulation of Fesitin Phytosomes by using independent and dependent variables

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Space Type	A:Fisetin Mg	B:Phosphotidyl Choline mg	C:Polaxomer P188 mg	Entrapment Efficiency %	Vesicle Size nm	Drug Release %
1	Factorial	572	1536	100	94	137.8	85.13
2	Axial	429	1152	167.045	95	177.6	82.7
3	Center	429	1152	125	93	162.4	91.6
4	Axial	429	1797.81	125	99	101.3	78.4
5	Factorial	286	768	100	95	151.1	73.38
6	Axial	188.504	1152	125	97	200.8	80.7
7	Center	429	1152	125	93	162.4	83.15
8	Center	429	1152	125	93	162.4	91.6
9	Factorial	572	1536	150	91	716.1	87.16
10	Factorial	572	768	150	94	208.7	88.65
11	Center	429	1152	125	93	162.4	91.6
12	Axial	429	506.192	125	91	196.4	72.42
13	Center	429	1152	125	93	162.4	91.6

14	Factorial	572	768	100	95	214.2	84.71
15	Factorial	286	1536	150	98	148.6	77.25
16	Factorial	286	1536	100	94	140.4	79.53
17	Axial	669.496	1152	125	94	254	80.54
18	Factorial	286	768	150	95	113.5	83.7
19	Center	429	1152	125	93	162.4	91.6
20	Axial	429	1152	82.9552	93	619.6	86.53

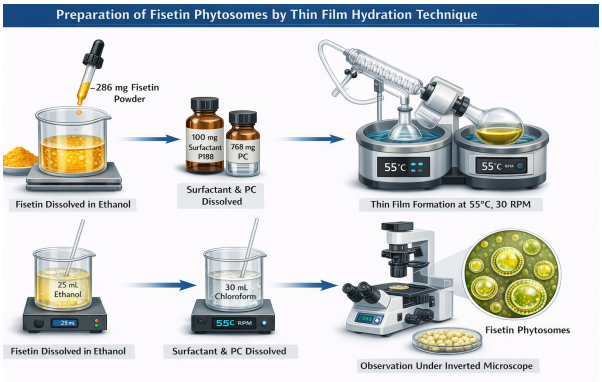


Fig:1Preparation of Phytosomes by Thin film Hydration Technique

Evaluation of Fisetin Phytosomes

Entrapment Efficiency:

The entrapment efficiency was determined by measuring the concentration of unloaded drug in the dispersion medium. The prepared SLN's samples were placed in 9 centrifuge tubes. Using a research centrifuge at 10000 rpm at 4°C for 15 mins, the Phytosomes were separated from the medium. The supernatant clear liquid was then diluted (if necessary) with buffer and the samples was measured by using UV visible spectrophotometer (SHIMADZU) at 362 nmentrapment efficiency can be calculated by using the formula[7]

$$\frac{\text{Total amount of drug used for the preparation} - \text{amount of unloaded drug}}{\text{total amount of drug used for the preparation}} \times 100$$

Particle Size and Zeta Potential: Particle size and Zeta potential was determined by using Horiba SZ100 at 250°C. The samples were diluted and probe sonication avoid possible interference during the measurement means of results.[7]

Surface Morphology by Transmission electron microscopy:

A drop of sample is placed on a piece of Para film, the carbon coated copper grid wait for 5-10 minutes, and drain the excess with help of filter paper, wash with distilled water and stained with 2% uranyl acetate dry observed under transmission electron microscopy at various magnification (modelHitachi-H-7500)[8].

***In-vitro* Diffusion Studies:**

The *in vitro* diffusion studies were carried out in diffusion cell using 6.8 pH Phosphate buffer as dissolution medium and maintain 37±0.50°C with mild magnetic stirring. At regular intervals of 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8 hr, 9hr, 10hr, 11hr,12hrs and 4 ml of sample was withdrawn and replace with 4 ml of 6.8 pH phosphate and samples were analyzed at 362 nm by using UV spectrophotometer and calculate the cumulative percent drug release and values in table[8].

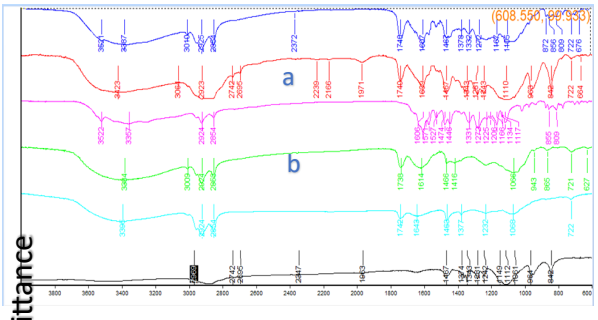


Fig:2 a.) FTIR of Spectra Fesitin Phytosome formulation, b) FTIR spectra of Fesitin +Lecithin+Kolliphor P188 Mixture, c) FTIR spectra of Fesitin +Lecethin Mixture. d) FTIR of spectra of Lecethin spectra, e) FTIR spectra of Kolliphor P188, f) FTIR spectra of Fesitin

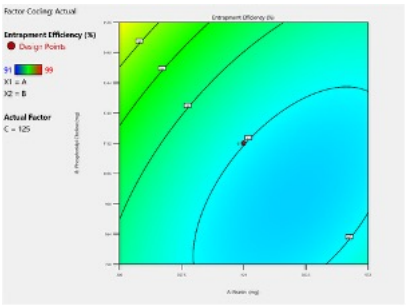


Fig:3a) Counter Plot of Entrapment Efficiency
Fig 3b)3D graph of Entrapment Efficiency

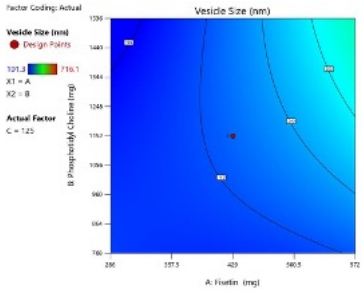


Fig:3c) Counter Plot of Particle size
Fig:3d) 3D graph of Particle size

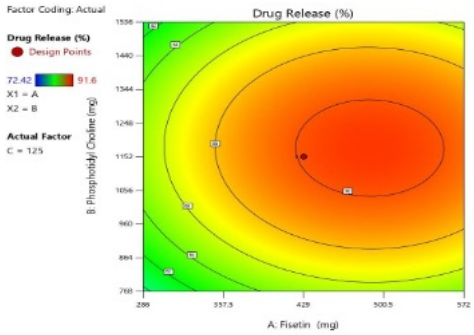


Fig:3e) Counter Plot of Drug release
Fig:3f) 3D graph of Drug release

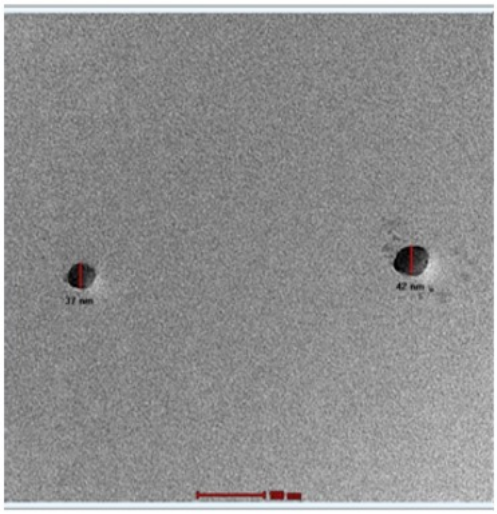


Fig:4a) TEM Image of F1 Formulation
Fig:4b) TEM Image of F1 Formulation

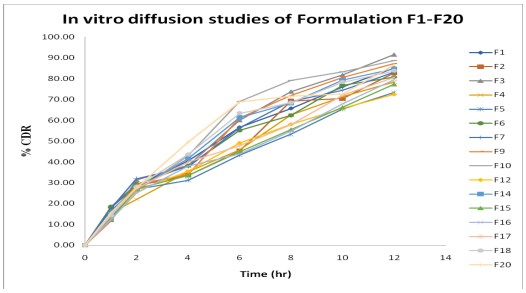
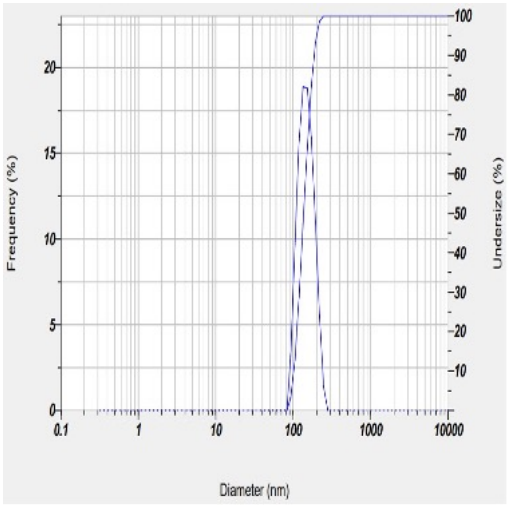
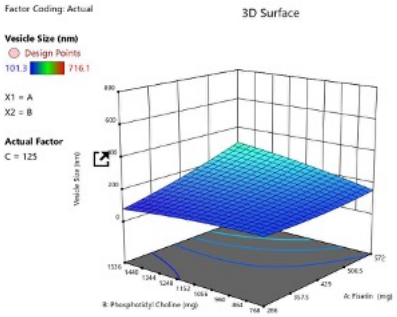
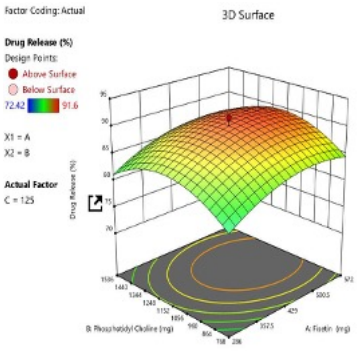
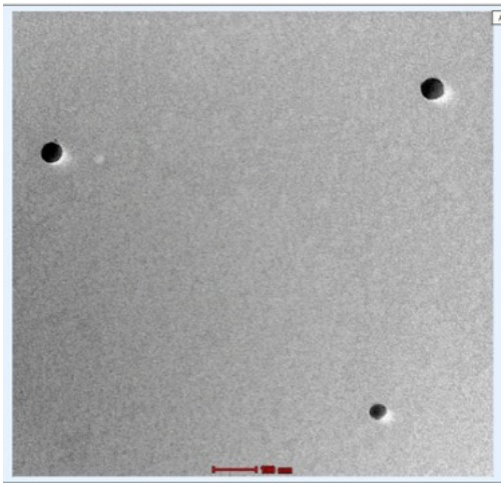


Fig:5 *In vitro* drug release studies of Fisetin Phytosome formulationsF1-F20









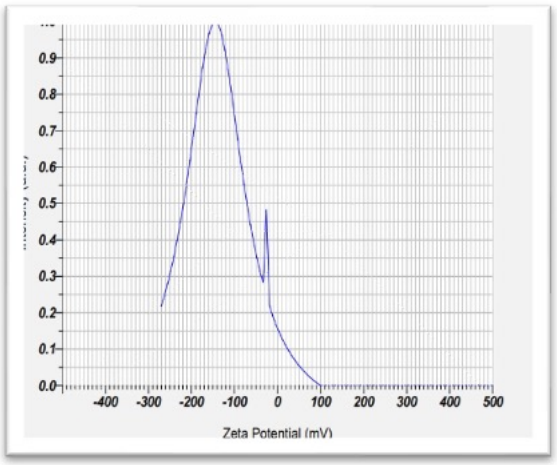
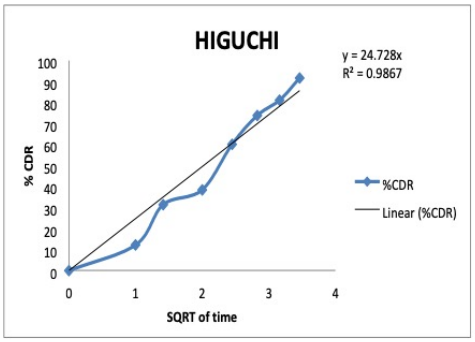


Fig: 6a) Particle size of F2 formulation
Fig :6b)Zetapotential of F2 formulation

Fig:7a) Zero order kinetics
Fig7b) First order kinetics



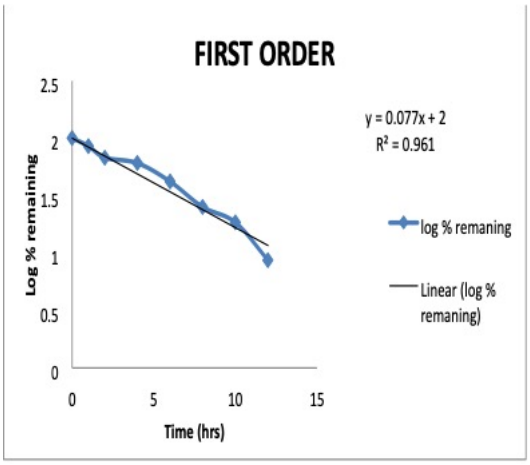


Fig: 7c).Higuchi model
Fig :7d) Korsmeyer- peppas model

Discussion:

Fisetine Loaded Phytosomes were successfully Prepared by Thin film hydration technique using Rota Evaporator. Phosphotidylcholine,as a Lipid, Kolliphor P188 as stabilizer. In order to determine the linearity of Fisetine in 6.8 pH phosphate buffer, calibration curve was constructed. The standard graph was plotted between concentration Vs absorbance which was displayed in figure. From the results, it was concluded that the Fisetine obeys the Beer lambers law. The value of R2 is 0.999 which shows that the plotted line was passing through the origin and is found to be linear.

The FTIR spectra for pure Fisetine were given in the figure:2 From the graphs it is noticed that the absorption band at 3521.74 cm-1 , 2924.38 cm-1, 1606.13 cm-1, 1206.83 cm-1, 855.41 cm-1 indicate presence of O-H, Ar C-H, C=O, C-O-C, C-H stretching vibration corresponding to Fisetine. In Phosphotidylcholine ,Kolliphor P188, peaks are observed at 3394.81 cm-1,
2923.80 cm-1, 2286, cm-1 1741.52 cm-1, 1231.80 cm-1, 842.28 cm-1 Indicate presence of methyline ,carbonyl, Ester, Alcohols, Aliphatic ether and there were no interactions between drug and excipients. Due

to slightly shift in the peak, we have concluded that drug and excipients are compatible with each other.

Developed formulations were evaluated for determining the parameters such as Entrapment efficiency, Particle size, Zeta potential which are mentioned in the table:1. Particle size distribution of F5and Fig:.6a) and 6b) Particle size distribution of F11 The particle size and Zeta potential of all formulations was found within the range. TEM was performed for determining the surface morphology and shape of Fesitine Phytosomes from the fig:4a) and 4b). TEM micrograph of F1 revealed that the Phytosomes are in spherical shape and variable in size. EE was performed for all formulations; among all formulations the highest amount (92.4%) of the drug was entrapped in the lipid core. The *in vitro* drug release of all formulations for 12hrs was performed. Release kinetics for all formulations are shown in the graph Fig:5and Fig:7. *Invitro* release kinetics of F1-F20. Among all the formulations F3 has the *in vitro* release of 91.65% and it follows the non fickian transport mechanism.

Optimization results:

Analysis of data: Design expert software (13.0.5.0). was used to fit the all responses into quadratic and linear models. Independent and dependent variables which are inserted into the software are displayed in the table.1.Independent and dependent variables The normal, predicted vs actual, Counter, 3D and Cubic plots for all responses of all formulation factors are shown in the Fig 3.By applying constraints on dependent variables and independent variables, optimized formulation was obtained. The optimized formulation which contains the concentration of Fisetin 429mg, Phosphotidyl Choline 1152mg and Kolliphor P188 125 mg was obtained from the software. All the physiochemical Parameters were obtain by the statistically optimized formulation. For the optimized formulation, entrapment efficiency, particle size, zeta potential and *in vitro* drug release was performed. The entrapment efficiency of optimized formulation was found to be 92.4 %. Particle size162.4 nm and zeta potential of optimized formulation was obtained within the range. *In vitro* drug release of optimized formulation (91.36) demonstrated that the percent CDR values are in close proximity with the predicted values of the models. The differencebetween predicted and experimental values is expressed in the form of percentage. The difference between predicted and experimental values is known as relative error (%) which confirms the validity and predictability of the model.

Conclusion

Central composite design was used to optimize the Fisetin loaded Phytosomes using Phosphotidyl choline as lipids and Kolliphor 188 as surfactant and stabilizer. By inserting different concentrations of factors and responses into design software, optimized formulation was obtained. The Optimized Phytosome formulation with Fisetin 429mg, Phosphotidyl Choline 1152mg and Kolliphor P188 125 mg showed the better release within 12hrs. The Vesicle size and morphological study by TEM analysis reveals nano size range and Zetapotential measurement shows the colloidal stability of ofphytosomes. The optimized formulation whose release kinetics follows Zero order kinetics and represents non fickian mechanism. So finally from all the results it was concluded that the optimized Phytosome formulation is the effective herbal carriers for improving the oral bioavailability of Fisetin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

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